

Learning objectives for the LCI training

During the LCI training, we provide teaching as well as scientific advice.

A. Scientific advice

We discuss with you potential sample preparation improvements to minimize sample to sample variability and ensure extraction of reliable data. We help you define your experimental goals in terms of which metrics need to be extracted from the image.

B. Training

At the end of the training, you should be able to independently perform the following actions:

1. Equipment operation

☐ Turn the equipment on/off according to the recommended procedure that leaves the equipment and the whole area clean.

☐ Mount and find your sample according to the recommended procedure in a way that is safe for the sample and the equipment.

☐ Choose appropriate acquisition parameters to adjust the acquisition speed and signal to noise ratio to the requirements of your scientific question and justify your choice.

☐ Illumination power

☐ Exposure time

☐ Gain

☐ Bit depth

☐ Choose appropriate optical and digital resolutions to adjust the image sampling to the requirements of your scientific question and justify your choice.

☐ Objective

☐ Pixel size

☐ Extra magnification lens

☐ Binning

☐ Choose appropriate multidimensional parameters to be able to reliably answer your scientific question and justify your choice.

☐ Multiple xy positions

☐ Overview/tiling

☐ z stacks

☐ Multiple channels

☐ Timelapse

☐ Define a focus strategy to initially set the focus then maintain it to ensure reliable and reproducible data acquisition.

☐ Display the image in a way that allows viewers to see the details relevant to the scientific question.

☐ Transfer the data according to the recommended safe procedure.

☐ Write the Material and Method section for your imaging experiment to enable reproducibility by other researchers.

2. Elimination of artifacts: bleedthrough, saturation and optical aberrations.

☐ Assess the efficiency of imaging and the potential for bleedthrough for the fluorophores in your sample when using the microscope selected for the training.

☐ Identify saturation/underexposure in acquired images, explain the consequence of both and explain which options are available to eliminate these artifacts.

☐ Identify and eliminate refraction index mismatch and chromatic aberration artifacts.

3. Optional items, depending on the experimental needs.

☐ Set the incubator for a live imaging experiment.

☐ Adjust the Koehler illumination to optimize transmitted light imaging.

☐ Run an acquisition pipeline established by another LCI user.

☐ Set the parameters for unmixing.

☐ Adapt a JOBS pipeline

☐ Acquire and process DeepSIM images. Recognize artifacts and adjust the DeepSIM parameters to eliminate them.

☐ Stitch tiled images post-acquisition for light sheet imaging.

☐ Deconvolve images and explain how to recognize and eliminate deconvolution artifacts.

☐ Align the NSPARC detector and set the acquisition parameters to eliminate the Pile up effect.

☐ Perform laser microdissection.

☐ Perform micropatterning and microfabrication.